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Mode of Action: Developmental Thyroid Hormone Insufficiency—Neurological Abnormalities Resulting From Exposure to Propylthiouracil

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Because thyroid hormone is essential for normal brain development before and after birth, environmental chemicals that interfere with thyroid hormone signaling can adversely affect brain development. Adverse consequences of thyroid hormone insufficiency depend both on severity and developmental timing, indicating that environmental antithyroid factors may produce different effects at different developmental windows of exposure. Mechanistic studies can provide important insight into the potential impact of chemicals on human thyroid function, but relevance to humans must be systematically evaluated. This kind of analysis depends on data sets that include information about animals and humans. The drug 6-*n*-propyl-2-thiouracil (PTU) is used in animals to experimentally manipulate serum thyroid hormone levels, and in humans to treat patients, including pregnant women, with Graves' disease. A systematic analysis of the mode of action (MOA) of PTU in rats and in humans discloses similar modes of action. While the analysis predicts that PTU doses that produce thyroid hormone insufficiency in humans would adversely affect the developing brain, careful monitoring of PTU administration in pregnant and lactating humans keeps infant serum thyroid hormone levels within the normal range.

Keywords Brain Development, Human Relevance, Propylthiouracil, Thyroid Hormone

Thyroid hormone (TH) is essential for normal human brain development, both before (Cao et al., 1994; Chan and Rovet, 2003; Haddow et al., 1999) and after (Heyerdahl and Oerbeck, 2003; Oerbeck et al., 2003; Rovet, 2002) birth. For this reason, environmental factors that interfere with thyroid function have the potential to cause TH insufficiency to such an extent that adverse effects on the offspring may result. Considering the large number of environmental chemicals known to interfere with thyroid function (Brucker-Davis, 1998; Howdeshell, 2002), it is important to establish an approach to evaluate the ability of these chemicals to produce neurotoxic effects by reducing circulating levels of TH. Animal studies are the only mechanism for rigorous experimental analysis of environmental chemicals. Therefore, it is important to perform a systematic analysis of the human relevance of experimental data, and this kind of analysis can best be applied to data sets that include information about animals and humans. However, there are few situations in which both humans and animals are exposed to known amounts of a thyroid toxicant so that comparisons can reasonably be made to determine the relevance of animal studies to human health. The drug 6-*n*-propyl-2-thiouracil (PTU) has been intensively used in animals to experimentally manipulate serum thyroid hormone levels, and in humans to treat patients, including pregnant women, with Graves' disease (Cooper, 2003). Comparing the effects of PTU in both animals and in humans can provide insight into the relevance of animal models to human health (Cohen et al., 2003).

PTU is well known to reduce circulating levels of T_4 and T_3 and to increase circulating levels of thyroid-stimulating hormone (TSH) (e.g., Frumess and Larsen, 1975; Sato et al., 1976). It has been extensively used in basic research programs using experimental animals to identify the role of thyroid hormone in brain development. In addition, the ability of PTU to reduce circulating thyroid hormone levels has been exploited in the treatment of hyperthyroidism in humans, including in pregnant

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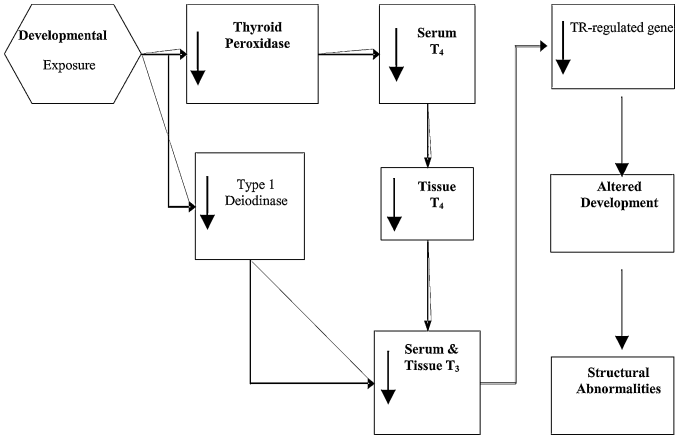

FIG. 1. Hypothesized MOA for PTU-induced neurotoxicity in animal development.

and lactating women (Mestman, 1998). As a chemical that may be prototypical in its antithyroid effects, we evaluated information on this drug as it may inform analyses of weak antithyroid agents that may exert a similar MOA.

There is a high degree of confidence that the animal MOA for PTU-induced developmental neurotoxicity is plausible in humans. This MOA includes inhibition of thyroid hormone synthesis and the subsequent reduction in circulating levels of thyroid hormone, which compromises the developing brain. However, because human exposure is necessarily under strict medical supervision, the doses of PTU used are titrated such that circulating levels of thyroid hormone are reduced to within the normal range; thus, neurotoxicity is not likely to occur in humans.

I. IS THE WEIGHT OF EVIDENCE SUFFICIENT TO ESTABLISH THE MOA IN ANIMALS?

Key Events

The flow charts given next in Figure 1 and Table 1 outline key events by which PTU exerts an effect on brain development in laboratory animals. Key events for which specific data are available are listed in **bold type.**

1. ***Thyroperoxidase inhibition***. Thyroperoxidase (TPO) is a multisubstrate enzyme that reacts first with hydrogen peroxide, forming an oxidized enzyme. This "activated" enzyme then oxidizes iodide, the second substrate, to an enzyme-bound "active iodine," transferable to tyrosyl residues on thyroglobulin (Tg) (Davidson et al., 1978). The thioureylene drugs, including PTU, methimazole (MMI), and thiouracil, can inhibit the ability of thyroperoxidase to activate iodine and transfer it to Tg (Davidson et al., 1978). This key event ultimately decreases circulating levels of thyroid hormones T_4 and T_3.
2. *Type 1 deiodinase inhibition (D1).* PTU also inhibits the Type 1 5′-deiodinase (Ortega et al., 1996) that converts T_4 to T_3 in peripheral tissues such as liver. PTU binds covalently to the Type 15′-deiodinase, forming a seleno-sulfide moiety and irreversibly inhibiting the enzyme's activity (Leonard and Rosenberg, 1980; Visser et al., 1979; Yamada et al., 1981). Because circulating levels of T_3 are largely controlled by the action of D1, it is likely that PTU causes a decrease in circulating levels of T_3 largely by inhibiting D1.
3. ***Reduction in thyroid hormone synthesis***. PTU-induced thyroperoxidase inhibition reduces the synthesis of nascent thyroid hormone, including both T_4 and T_3, as evidenced by the reduction in incorporation of ^{125}I into thyroglobulin (Yi et al., 1997).
4. ***Serum T_4 is reduced***. The reduction in thyroglobulin iodination causes a dose-dependent decrease in circulating levels of both T_4 and T_3 (Cooper et al., 1983). In addition, because approximately 80% of circulating T_3 is generated from the action of type 1 deiodinase (Cooper et al., 1983), the PTU-induced reduction in serum T_3 is likely to be due to the combined action of PTU on TPO and on D1 inhibition.
5. ***Tissue levels of thyroid hormone are reduced***. The relationship between serum concentrations of thyroid hormones and tissue levels was evaluated in adult rats by Oppenheimer et al. (Oppenheimer, 1983). This work established that tissue levels of thyroid hormone, especially in brain, decreased relative to serum T_4 and T_3 concentrations. This is supported empirically by work in developing animals of Morreale de Escobar and Porterfield (Calvo et al., 1992; Morreale de Escobar et al., 1987, 1990; Porterfield and Hendrich, 1992, 1993; Porterfield and Stein, 1994). Moreover, recent work indicates that the relationship between serum and tissue levels of hormones is somewhat dependent on the brain area (Broedel et al., 2003).
6. *Altered expression of thyroid hormone-responsive genes.* Circulating levels of thyroid hormone are correlated with changes in the expression of specific genes in the developing brain (Bernal, 2002; Bernal and Guadano-Ferraz, 2002). These thyroid hormone-responsive genes encode proteins that are important in modulating developmental events in the brain, though the exact developmental events have not been fully explored (Zoeller, 2003, 2004).
7. ***Permanent structural abnormalities in the brain***. Effects of thyroid hormone insufficiency on specific developmental events lead directly to effects on brain structure. These include, but are not limited to, effects on the size and composition of the corpus callosum (Berbel et al., 1993, 1994; Gravel and Hawkes, 1990; Gravel et al., 1990), cerebellum (Koibuchi and Chin, 2000; Madeira et al., 1988a, 1988b; Singh et al., 2003), hippocampus (Madeira et al., 1988c, 1991), and others.
8. ***Behavioral abnormalities***. PTU-induced hypothyroidism affects a variety of behaviors; the pattern of behavioral disturbance depends on the developmental timing of PTU administration (Zoeller and Rovet, 2004). Brosvic et al. (2002) found that animals prenatally exposed to PTU exhibited a number of complex cognitive and motor deficiencies. Friedhoff et al. (2000) found that prenatal thyroid hormone insufficiency also

TABLE 1
Key events in the animal MOA

Key event	Evidence in animals	References
Inhibition of TPO	YES: In vitro evidence for PTU inhibition of TPO activity and subsequence molecular mechanisms.	Davidson et al., 1978; Nagasaka and Hidaka, 1976
Inhibition of type 1 5′-deiodinase	YES: In vitro evidence for PTU inhibition of type 15′ -deiodinase	Leonard and Rosenberg, 1980; Visser et al., 1979; Yamada et al., 1981
Reduction in thyroid hormone synthesis	YES: In vivo evidence that PTU inhibits the incorporation of ^{125}I into Tg.	A direct measurement: Francis and Rennert, 1980
Reduction in serum T_4	YES: A great many studies demonstrate that PTU reduces serum T_4 in animals.	An important example: Cooper et al., 1983
Decreased tissue level of T_4 and T_3	YES: Several studies have shown empirically that tissue levels of thyroid hormones are proportional to serum hormone level; manipulated by PTU.	Oppenheimer, 1983; Morreale de Escobar et al., 1987; 1990; Calvo et al., 1992; Porterfield and Hendrich, 1992, 1993; Porterfield and Stein, 1994; Broedel et al., 2003
Altered expression of thyroid hormone-responsive genes in the developing brain	YES: There are a very large number of studies showing that a reduction in thyroid hormone levels alters gene expression in the developing brain; many of these studies use PTU to modify thyroid hormone levels; however, it is not clear in most of these studies if the observed changes in gene expression are causally linked to production of adverse effects.	A comprehensive review: Bernal et al. 2003
Permanent structural defects in the brain	YES: There is a large literature showing that PTU-induced thyroid hormone insufficiency produces structural defects in the developing brain. However, the causal relationship among the key events described here is not often demonstrated.	Berbel et al., 1993, 1994; Gravel and Hawkes, 1990; Gravel et al., 1990; Koibuchi and Chin, 2000; Madeira et al., 1988a, 1988b; Singh et al., 2003; Madeira et al., 1988c, 1991
Permanent changes in behavior	YES: There is a very large literature on the effects of thyroid hormone insufficiency, produced by PTU, on behavior	Akaike et al., 1991; Brosvic et al., 2002; Friedhoff et al., 2000; Gordon, 1997

produced hypoactivity in open field tests, whereas postnatal thyroid hormone insufficiency produced hyperactivity in these tests (Akaike et al., 1991).

Other Evidence

A very large literature documents the effects of PTU-induced thyroid hormone insufficiency on developmental endpoints in the brain (Anderson et al., 2003; Bernal et al., 2003; Thompson and Potter, 2000). Broadly, these endpoints may be categorized as measures of histogenesis, neurochemistry, gene expression, or behavior. Examples of PTU effects on these categories of developmental events are provided next, but a great variety of methods have been employed to reduce circulating levels of thyroid hormone during development, including the use of other goitrogens (e.g., methimazole), surgical thyroidectomy, and ^{131}I, and regardless of the method of thyroid hormone suppression, the effects on brain development are largely the same. However, one general trend emerging from the literature is that the specific effects resulting from thyroid hormone insufficiency depend on the developmental stage at which the insufficiency occurs (Zoeller and Rovet, 2004).

Dose-Response Relationships

A number of reports have focused on dose-response relationships between PTU, circulating levels of thyroid hormone, and thyroid histopathology in adult rats (Marty et al., 1999, 2001a, 2001b; O'Connor et al., 2002). The goal of these studies was to characterize the relationship between serum thyroid hormone suppression, reflexive TSH increase, and histopathological measures within the thyroid gland that may be indicative of a precancerous state. This logic has been well characterized

by Capen (1994, 1997b) and will not be further considered here. Brosvic et al. (2002) characterized the dose-related effect of PTU on maternal serum thyroid hormone and various behaviors in their pups. In a seminal study, Cooper et al. evaluated the dose response of PTU on thyroid function in the young adult rat (Cooper et al., 1983). Goldey et al. (1995) reported on a dose-dependent relationship between PTU, circulating levels of thyroid hormone, and hearing in the dams' progeny. In each of these studies, PTU was related to serum thyroid hormone in a dose-dependent manner. However, a dose-response relationship does not always exist between the severity of thyroid hormone insufficiency and the severity of affected endpoints of gene expression or development in the brain. Few studies have addressed this issue.

Strength, Consistency, and Specificity of Association of PTU-Induced Thyroid Hormone Insufficiency and Neurodevelopment

Although few laboratories have focused on PTU as a drug, many laboratories throughout the world have employed PTU to study the effects of experimentally induced thyroid hormone insufficiency. Without exception, these reports document the relationship between PTU treatment, serum thyroid hormone insufficiency, and measures of neurological impairment (Bernal, 2002; Bernal and Guadano-Ferraz, 2002; Bernal et al., 2003).

Temporal Association and Reversibility

Cooper et al. (1983) characterized early the reversibility of PTU exposure on thyroid function in the adult rat. These investigators found that 50% suppression of thyroid protein-bound iodine (PBI) occurred at a PTU concentration in the drinking water of 0.0005% (ED50), with concomitant serum and thyroid PTU levels of 0.3 μg/ml and 300 ng/thyroid, respectively. After 1 month of PTU, serum T_4 values were lower than after 1 week of treatment for all PTU concentrations, but values for the other measures of thyroid function were similar to those observed after 1 week of treatment at comparable PTU dosage. The PTU dose-response curve for thyroid PBI (protein-bound iodine, an early measure of thyroid hormone) at 1 month of treatment was similar to that seen after 1 week, with an ED50 of 0.0004%. After discontinuation of PTU treatment, PTU disappeared from serum in a biexponential fashion, with an early rapid distribution phase ($t_{1/2}$ = approximately 4 h) and a second slower elimination phase ($t_{1/2}$ = approximately 2.6 days). In the thyroid, an initial increase in PTU content was seen up to 18 h after PTU withdrawal; thereafter, thyroid PTU declined linearly, with a $t_{1/2}$ of 1.4 days in both groups. After PTU withdrawal, thyroid PBI recovered with a $t_{1/2}$ of 1.09 days after 1 week on PTU, but recovery was prolonged ($t_{1/2} = 2.8$ days) after 1 month of treatment. Log thyroid PTU and log thyroid PBI were linearly related after PTU withdrawal ($r = .97$; p less than .001) after 1 week but not after 1 month. Serum T_4 and serum T_3 remained below control values for 2 days following PTU withdrawal, but then rapidly normalized, with T_3 values rising transiently above the control value. This rebound occurred at a time when PTU was still present within the thyroid and before thyroid PBI had returned to baseline. These data indicate a close inverse relationship between PTU dose and both thyroid hormone biosynthesis and peripheral T_4 deiodination.

The temporal relationship between thyroid hormone insufficiency and adverse effects on brain development has been articulated well by Howdeshell (2002). Few studies have focused on the temporal relationship between the onset of exposure to PTU and the onset of key events in the MOA proposed here. PTU exposure likely has a rapid effect on TPO inhibition, but the precise temporal relationship between TPO inhibition produced by PTU and Tg iodination is not well characterized. Other dietary TPO inhibitors can reduce TPO activity within minutes in vitro (Doerge and Chang, 2002); thus, TPO inhibition may be a rapid response to PTU. Once the dose of PTU is such that inhibition of Tg iodination begins, the temporal relationship between toxicant (i.e., PTU) exposure and circulating levels of thyroid hormone will depend on the storage capacity of the thyroid gland and the half-life of T_4 in the serum.

Biological Plausibility and Coherence of the Database

A very clear relationship exists between the MOA of PTU (i.e., inhibition of thyroid peroxidase and D1) and circulating levels of thyroid hormones and thyrotropin. Moreover, there is good evidence that elevated levels of TSH resultant from PTU exposure can lead to thyroid carcinoma in rats (Capen, 1997a). The considerable literature on the role of thyroid hormone on brain development has used PTU as the chemical of choice for reducing circulating levels of thyroid hormones (Bernal, 2002).

Alternate Modes of Action of PTU

No MOA other than reducing serum TH has been generally proposed to explain PTU's ability to affect brain development. However, alternate mechanisms may exist. For example, a recent study demonstrates that PTU can exert direct actions on the activity of the neuronal isoform of nitric oxide synthase (NOS) (Wolff and Marks, 2002). Considering the importance of neuronal NOS in brain development and in neuronal plasticity (Blackshaw et al., 2003), this direct action of PTU may influence brain development. Moreover, in view of the relationships between a variety of peroxidase enzymes and NOS activity (Abu-Soud et al., 2001), PTU could influence peroxidases other than thyroperoxidase with deleterious effects. This has not been tested.

In addition, PTU could cause adverse effects on the developing brain by reducing serum T_4, which would interfere with T_4's ability to exert actions independent of the thyroid hormone receptor. Although it is generally held that the effects of thyroid hormone are mediated by TR regulation of target gene transcription in the nucleus, thyroid hormones (T_4, T_3, rT_3) clearly exert important effects on development and physiology through nongenomic mechanisms (Davis and Davis, 1996, 2002; Davis et al., 2002; Shibusawa et al., 2003). Early evidence for

the nongenomic effects of thyroid hormone include the lack of dependence on nuclear TRs and the rapid onset of action (typically seconds to minutes), utilization of membrane-signaling pathways, typically involving kinases or calmodulin, that have not been implicated in direct TR function (Yen, 2001). Thyroid hormone is known to directly influence the activity of ^{21}Ca-ATPase, adenylate cyclase, and glucose transporters (Yen, 2001).

Direct, nongenomic effects of thyroid hormone on mitochondria (Wrutniak-Cabello et al., 2001) may also be important contributions to the physiological actions of thyroid hormone. A number of studies report rapid actions of T_3 injections into hypothyroid rodents on oxygen consumption and oxidative phosphorylation measured in mitochondria isolated from hepatocytes (Palacios-Romero and Mowbray, 1979; Sterling, 1986), which may affect brain development. Leonard and Farwell (1997) demonstrated that thyroid hormone can catalyze actin polymerization in an in vitro system of primary astrocytes isolated from rat cerebellum. This is important for several reasons. First, regulated actin polymerization is important in neurite outgrowth and cell motility (Dent and Kalil, 2001). Second, this activity was affected by T_4 and rT_3, but not T_3 (Farwell and Dubord-Tomasetti, 1999; Leonard and Farwell, 1997). T_4 and rT_3 can stimulate vesicular transport in cells by activating myosin V motors (Stachelek et al., 2000, 2001). These effects are not mediated by the TRs (TRα1, TRβ1, TRβ2).

Conclusions—Assessment of Postulated MOA in Animals and Statement of Confidence

A considerable amount of information provides a high degree of confidence that PTU reduces circulating levels of thyroid hormone by inhibiting TPO activity within the thyroid gland. Dose-response studies performed in vitro with recombinant or extracted TPO provide a high degree of certainty that the inhibitory effect of PTU on this enzyme can block the organification of thyroglobulin and thus, block thyroid hormone synthesis. Moreover, it is clear that PTU-induced hypothyroidism decreases hormone levels in the developing brain, with consequent alterations in TR-responsive genes, disruption of structural development, and functional outcomes.

II. ARE KEY EVENTS IN THE ANIMAL MOA PLAUSIBLE IN HUMANS?

Concordance Analysis of Key Events

Analysis of the relevance of animal data to humans has two principal components. First, we review the data derived from humans that relate to the MOA as defined in animals. Second, we review the literature, though much less robust, that relates to the relative sensitivity of humans to PTU compared to animals. See Table 2 for a summary.

1. *Thyroperoxidase inhibition.* PTU inhibits human thyroperoxidase, purified from human thyroid (Nagasaka and Hidaka, 1976; Sugawara, 1985). Although there is not a great deal of work to confirm that the human TPO enzyme is inhibited by PTU by the same mechanism as that which inhibits rodent TPO, there is every reason to expect that this is the case, especially considering the conservation among mammalian thyroid peroxidases (Taurog, 1999).
2. *Type 1 deiodinase inhibition.* In vitro work has shown that PTU blocks the human Type 1 5′-deiodinase (Campos-Barros et al., 1996; LoPresti et al., 1989; Mandel et al., 1992).
3. *Thyroid hormone synthesis is reduced.* There are no formal studies on the ability of PTU to inhibit thyroid hormone synthesis in vivo, but it is clear that PTU inhibits TPO activity and reduces circulating levels of thyroid hormone in humans (see later discussion).
4. *Serum T_4 is reduced.* PTU is the drug of choice in pregnant women with hyperthyroidism (Mestman, 1998). The goal of therapy is to maintain serum free T_4 in the mother at the upper range of normal with the minimal dose of PTU (Mestman, 1998). These studies show that PTU inhibition of TPO in humans causes a reduction in serum T_4.
5. *Tissue levels of thyroid hormone are reduced.* There are no reports of direct measurements of tissue levels of T_4 in humans following PTU treatment. However, there is no evidence that the relationship between the level of serum thyroid hormone and tissue levels of thyroid hormone in humans is different from that in animals.
6. Altered expression of thyroid hormone-responsive genes. There are no studies that directly inform us about gene expression in the developing brain in response to PTU treatment in humans. However, in vitro studies of thyroid hormone regulation of human gene expression demonstrate the same relationship between hormone level and gene expression in humans compared to animals (Bernal et al., 2003).
7. *Permanent structural abnormalities in the brain.* It is clear that thyroid hormone insufficiency, caused by factors other than by PTU, will cause structural abnormalities in the developing brain. For example, structural and biochemical abnormalities are measurable by magnetic resonance imaging (MRI) in young children with congenital hypothyroidism (Gupta et al., 1995b; Jagannathan et al., 1998).
8. *Behavioral abnormalities.* There are no studies linking developmental exposure of PTU to psychomotor function. However, Momotani et al. (2000) report that some infants whose mothers took relatively high doses of PTU during pregnancy and lactation (up to 750 mg/day) exhibited elevated serum TSH. Although psychomotor tests were not performed, it may be postulated that these children would have exhibited measurable psychomotor deficits, based on the literature focused on congenital hypothyroidism (Heyerdahl, 2001; Heyerdahl et al., 1991; Heyerdahl and Oerbeck, 2003; Rovet, 2002).

III. TAKING INTO ACCOUNT KINETIC AND DYNAMIC FACTORS, IS THE ANIMAL MOA PLAUSIBLE IN HUMANS?

The animal MOA is plausible in humans (Table 2). PTU inhibits the human TPO in vitro (Sugawara, 1985), leading to

TABLE 2
Key events in the animal MOA and human relevance

Key event	Evidence in animals	Evidence in humans	References
Inhibition of TPO	YES: Both in vivo and in vitro evidence	YES: In vitro assay to demonstrate that effect on human TPO is similar to that of rodent	Sugawara, 1985
Inhibition of type 1 5′-deiodinase	YES: In vitro evidence for PTU inhibition of type 1 5′-deiodinase	YES: Ex vivo and in vitro demonstration that PTU blocks human type 1 5′-deiodinase	Campos-Barros et al., 1996; LoPresti et al., 1989; Mandel et al., 1992
Reduction in thyroid hormone synthesis	YES: Animal studies confirming this are prolific	NO: Although there are no direct studies of PTU effects on thyroid hormone synthesis in humans in vivo, all indications are that PTU inhibits synthesis	
Reduction in serum T4	YES: A great many studies demonstrate that PTU reduces serum T_4 in animals	YES: Clinical data demonstrate that PTU reduces maternal T_4 (in Graves' patients) and T_4 in neonates	(Atkins et al., 2000; Gardner et al., 1986; Mortimer et al., 1990
Decreased tissue level of T_4 and T_3	YES: Studies show that PTU causes a reduction in tissue T_3, T_4	NO DATA: We know of no studies to determine whether PTU in humans causes a reduction in T_4 and/or T_3 in brain tissue	
Altered expression of thyroid hormone-responsive genes in the developing brain	YES: Large numbers of studies demonstrate that PTU changes gene expression in the developing rodent brain	NO DATA	
Permanent structural defects in the brain	YES: Effects of developmental exposure on cerebellum, hippocampus, and cerebral cortex	YES/NO: No data available for PTU; mixed evidence in that children with congenital hypothyroidism exhibit neurochemical deficits by MRI	Gupta et al., 1995a
Permanent changes in behavior	YES: Effects of developmental exposure on motor function, learning, and memory	NO DATA: May be inferred by data on congenital hypothyroidism	

a decrease in circulating levels of thyroid hormones (Cooper, 2003), which is the basis of its use as an antithyroid drug in the treatment of Graves' disease. In addition, case reports indicate that PTU can reduce circulating levels of thyroid hormone in both the human fetus and neonate. For example, two case reports describe a fetal goiter observed by ultrasound imaging when the mother was treated for Graves' disease with PTU (Friedland and Rothschild, 2000; Ochoa-Maya et al., 1999). Presumably, the goiter was the result of PTU blocking thyroid function, which caused an increase in TSH, stimulating goiter formation. Reducing PTU dose to the mother caused the goiter to resolve in both cases. Likewise in the infant, if the PTU dose is too high for the mother, the infant may begin to exhibit lower circulating levels of thyroid hormone (Momotani et al., 2000). For these reasons, the exposure assessment component of the overall risk assessment process—for PTU, the clinically prescribed dose—is based on the large body of information provided by animal studies as well as these case reports.

However, larger studies have failed to identify neurological or cognitive deficits in children of women treated with PTU for Graves' disease (Burrow et al., 1968, 1978; Eisenstein et al., 1992; McCarroll et al., 1976; Messer et al., 1990). Thus, PTU does not appear to affect brain development in humans largely because the dose is titrated to bring circulating levels of thyroid hormone in Graves' disease patients to the normal range.

Interspecies Differences in Sensitivity to Thyroid Toxicants (PTU)

Despite the biochemical and endocrinological similarities between rats and humans, rats clearly exhibit reductions in serum thyroid hormone levels in response to PTU much more quickly than do humans (Cooper, 2003; Cooper et al., 1983; Gardner et al., 1986; Halpern et al., 1983). Thus, the relative sensitivity of rats compared to humans becomes an important issue in assessing the animal data for relevance to humans. Animal models confirm that developmental hypothyroidism produces adverse health outcomes. Current data suggest that the sensitivity of animal models to thyroid disruption depends on whether the health outcome being considered is cancer or cognitive development. There is good evidence that rats are more sensitive than humans to the production of thyroid tumors by thyrotoxicants that lower serum T_4 (Capen, 1998; DeVito et al., 1999). On the other hand, current animal models of the effects of thyroid hormone insufficiency on brain development have not been characterized in a manner that allows us to determine whether they are more or less sensitive than humans to TH insufficiency.

Two questions are important. First, to what extent must circulating levels of thyroid hormone be reduced before brain development is impaired, and is this greater or lesser in rats compared to humans? Second, to what extent must thyroid hormone *synthesis* be reduced before serum hormone levels are impacted to the extent that adverse consequences result, and is this greater or lesser in rats compared to humans? In short, the answer to both of these questions is that we do not know.

First, *to what extent must circulating levels of thyroid hormone be reduced before brain development is impaired, and is this greater or lesser in rats compared to humans?* Formal dose-response studies to answer this question have not been conduced in humans or animals. However, small reductions in circulating levels of thyroid hormone can cause cognitive deficits in humans. Haddow et al. (1999) reported that the children of women exhibiting overt or subclinical hypothyroidism exhibited measurable cognitive deficits at age 7–9 years, but these children did not exhibit evidence of low thyroid hormone levels at birth or later. This observation is well supported by a large literature correlating maternal thyroid status with cognitive development in the offspring in the absence of overt hypothyroidism in the child at birth or later (Chan and Rovet, 2003). In addition, small differences in circulating levels of thyroid hormone in children diagnosed with congenital hypothyroidism are associated with cognitive deficits in early adulthood (Heyerdahl and Oerbeck, 2003; Oerbeck et al., 2003). Thus, while it is not possible to state the extent to which thyroid function must be impaired to damage brain development, it is clear that small changes in thyroid function can result in damage. Although less well studied in experimental animals, there are several reports that small reductions in maternal thyroid status can affect fetal brain development (Auso et al., 2004; Dowling et al., 2000, 2001; Dowling and Zoeller, 2000; Lavado-Autric et al., 2003).

A significant impediment to answering this question in experimental animals is that we are not fully apprised of the indicators of thyroid function (i.e., total or free T_4 or T_3, ratios of these measures, measurements of binding proteins, etc.) that are most closely associated with deleterious effects on brain development (Zoeller, 2003). Therefore, even if dose-response studies had been performed, it is currently impossible to know what measures, or combination of measures, of thyroid function should be related to measures of brain development.

Second, *to what extent must thyroid hormone synthesis be reduced before serum hormone levels are impacted to the extent that adverse consequences result, and is this greater or lesser in rats compared to humans?* Given the same dose (normalized to body weight) of antithyroid compound, such as PTU, rats will exhibit a significant reduction in circulating levels of thyroid hormone sooner than humans. Although this kind of study has not been performed directly, differences in thyroid economy between rats and humans lead to this prediction. For example, the serum half-life of T_4 in humans is about a week (Chopra and Sabatino, 2000), but approximately a day in rats (Chanoine et al., 1992); therefore, a decrease in thyroid hormone synthesis would be manifested in reduced serum hormone levels in rats prior to humans. In addition, the adult human thyroid gland contains a significant amount of stored thyroid hormone—perhaps several months worth (Greer et al., 2002). However, rats and humans may be similarly sensitive to the ability of PTU to reduce thyroid hormone *synthesis*. Likewise, considering that a human neonate has a serum half-life of T_4 of around 3 days (Vulsma et al., 1989) and intrathyroidal stores of T_4 estimated to be less than 1 day worth (van den Hove et al., 1999), it would be predicted that human neonates will exhibit a decrease in serum thyroid hormone levels prior to that observed in adults. Thus, humans and rats may be similarly sensitive to the effect of PTU on thyroid hormone synthesis, with rats exhibiting effects on hormone levels in a shorter duration of exposure than in humans, though the ultimate effect may be the same.

However, humans are not exposed to PTU inadvertently; this drug is used clinically to control circulating levels of thyroid hormone in people with autoimmune-induced hyperthyroidism (e.g., Graves' disease) (Cooper, 2003). Thus, it is clear that PTU reduces circulating levels of thyroid hormone in humans, but the dose is titrated so that serum hormone levels are brought to within the normal range. If PTU were given to humans in doses that reduce circulating levels of thyroid hormone to below the normal range, it is predictable that neurological effects would occur. Infants or children with low circulating thyroid hormones, such as those suffering from dietary iodine deficiency or congenital hypothyroidism, have impaired neurological development (Boyages and Halpern, 1993; Boyages et al., 1989; Brown et al., 1939; Klein, 1980; Pharoah et al., 1976; Rovet, 1999a, 1999b). Children lacking adequate thyroid hormones during development exhibit a variety of neurological deficits, including difficulty making coordinated motor movements, speech and hearing impairments, and cognitive deficits. Importantly, the timing of

thyroid hormone insufficiency defines the neurological domain affected, and the severity of hormone insufficiency defines the severity of the neurological deficits (Zoeller and Rovet, 2004). Severe hypothyroidism leads to cretinism, most often observed in geographic areas of iodine insufficiency. This syndrome is marked by pronounced mental and physical retardation (Cao et al., 1994; Delange, 1996; DeLong et al., 1994).

IV. CONCLUSION—STATEMENT OF CONFIDENCE

The early key events of inhibition of TPO resulting in reduced circulating levels of thyroid hormone have been amply documented in animals and in humans. In addition, there is a high degree of concordance between observed effects of thyroid hormone insufficiency and brain development in experimental animals (primarily rats) and effects of thyroid hormone insufficiency and brain development in humans. However, there are few dose-response studies in experimental animals that would support strong conclusions concerning the relative sensitivity of animals and humans to PTU effects on brain development. Thus, there is a high degree of confidence that the animal MOA for PTU-induced developmental neurotoxicity is plausible in humans. However, this MOA is not likely to occur in humans. PTU is used clinically to reduce circulating T4 levels to normal in hyperthyroid patients. Therefore, unless PTU is over prescribed and T4 levels are reduced to below normal, no developmental neurotoxicity due to hypothyroidism should occur.

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